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Risk Assessment for *Clostridium perfringens* in Ready-to-Eat and Partially Cooked Meat and Poultry Products†

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ABSTRACT

An assessment of the risk of illness associated with *Clostridium perfringens* in ready-to-eat and partially cooked meat and poultry products was completed to estimate the effect on the annual frequency of illnesses of changing the allowed maximal 1-log growth of *C. perfringens* during stabilization (cooling after the manufacturing heat step). The exposure assessment modeled stabilization, storage, and consumer preparation such as reheating and hot-holding. The model predicted that assuming a 10- or 100-fold increase from the assumed 1-log (maximal allowable) growth of *C. perfringens* results in a 1.2- or 1.6-fold increase of *C. perfringens*–caused illnesses, respectively, at the median of the uncertainty distribution. Improper retail and consumer refrigeration accounted for approximately 90% of the 79,000 *C. perfringens* illnesses predicted by the model at 1-log growth during stabilization. Improper hot-holding accounted for 8% of predicted illnesses, although model limitations imply that this is an underestimate. Stabilization accounted for less than 1% of illnesses. Efforts to reduce illnesses from *C. perfringens* in ready-to-eat and partially cooked meat and poultry products should focus on retail and consumer storage and preparation methods.

Clostridium perfringens is a gram-positive, spore-forming, anaerobic bacterium that grows well on meat and poultry products and grows at relatively high temperatures (maximum growth rate occurs at about 45°C). It is ubiquitous in the environment, and raw meat may be contaminated with *C. perfringens* vegetative cells or spores. *C. perfringens* has been estimated to cause approximately 250,000 annual illnesses in the United States *(33)*. Illness caused by *C. perfringens* is usually a self-limiting diarrhea. Of all *C. perfringens* strains, approximately 5% are type A and capable of producing the illness-causing enterotoxin *(32)*. Such *C. perfringens* strains in ready-to-eat and partially cooked (RTE/PC) meat and poultry products were the focus of this risk assessment.

During the production of RTE/PC foods, a heat treatment is applied to raw product. *C. perfringens* vegetative cells and other bacteria present in the raw product are killed by this heating. *C. perfringens* spores, on the other hand, are not killed by such heating and may be activated to germinate, becoming vegetative cells. By removing competitive bacteria, the heat treatment can thus create a favorable environment for the growth of spore-forming bacteria like *C. perfringens*. During the postcooking cooling period known as "stabilization," *C. perfringens* vegetative cells may germinate, outgrow, and multiply to levels that could cause illness if time and temperature permit. It is this growth that could allow *C. perfringens* to become a food safety hazard.

To limit the growth of *C. perfringens* on meat and poultry products, the Food Safety Inspection Service (FSIS) published a final rule that established a performance standard for *C. perfringens* during production of some RTE/PC foods. The performance standard is based on FSIS microbiological product surveys in which samples were found to contain approximately 10^4 cells of *C. perfringens* per g *(51)*. However, this survey did not distinguish between vegetative cells and spores; any *C. perfringens* detected was assumed to be spores. Using the Centers for Disease Control and Prevention (CDC) criterion of $\geq 10^5$ cells of *C. perfringens* per g in food for incriminating *C. perfringens* as the causative agent of foodborne illness, the Agency set a performance standard requiring that multiplication of *C. perfringens* be limited to a maximum of 1 log for a subset of RTE/PC products *(48)*. In 2001, FSIS published a proposed rule to extend this performance standard to all RTE/PC products *(50)*.

In light of comments received on the proposed rule, we performed a quantitative risk assessment to evaluate the likelihood of illness from RTE/PC products contaminated by *C. perfringens (50)*. The purposes of this risk assessment were to evaluate (i) the relative effect on the annual incidence of illnesses of changing the allowed maximal growth of *C. perfringens* during stabilization of RTE/PC and (ii) the uncertainty in the size of any such effect.

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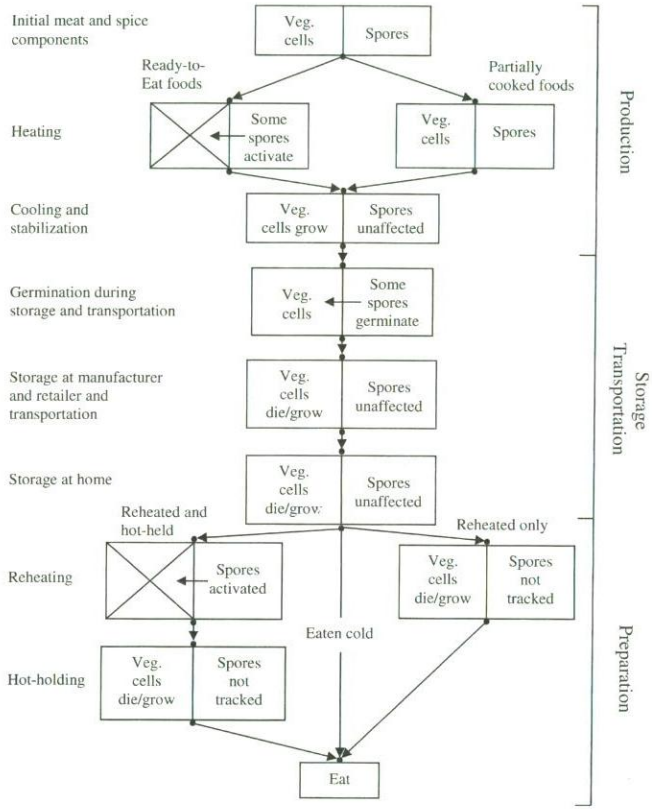

FIGURE 1. *Flow chart for modeling survival and growth of* C. perfringens *in RTE and partially cooked meat and poultry products.*

MATERIALS AND METHODS

To evaluate the effect of stabilization on *C. perfringens* foodborne illness, this risk assessment tracked the level of *C. perfringens* vegetative cells and spores in individual RTE/PC food servings through processing, storage, and preparation by using a computer model that can be envisioned as a sequence of modules (Fig. 1). The number of type A enterotoxin-positive *C. perfringens* vegetative cells and spores on a serving of RTE/PC product following the initial heat treatment and stabilization was estimated in the processing module. Results from the processing module were inputs for the storage module, wherein the number of *C. perfringens* vegetative cells and spores following retail and consumer storage was estimated. This was followed by the preparation module, which tracked the number of *C. perfringens* vegetative cells and spores following cooking and hot-holding of the food servings. The output of the exposure assessment was used with a dose-response curve to estimate the number of diarrheal illnesses resulting from the ingestion of *C. perfringens* vegetative cells.

Information and data for the development of this risk assessment model were obtained through Web-based searches of the scientific literature, citation searches, and backtracking through reference lists, together with industry and government data. Unpublished survey data were obtained through personal correspondence. In the mathematical modeling, variabilities among people, *C. perfringens* isolates, and food servings were represented to the extent possible by probability distributions. The parameters of these distributions were estimated from available data, although some data were used directly as empirical distribution functions. The uncertainty in parameter estimates was also represented by probability distributions. In most cases, parameters, their uncertainties, and correlations between them were simultaneously estimated from all relevant available data by using likelihood methods on subsidiary models considered to be representative of the experiments that generated the data; and all these estimates were used in the risk assessment modeling. In cases where no formal analyses of data were possible, variability distributions or fixed values were chosen ad hoc to represent what data were available, and the sensitivity to such choices of the results obtained was evaluated. The computer implementation was a two-dimensional Monte-Carlo model that used a set of general-purpose Monte-Carlo modeling routines written in object-oriented Pascal. Full details, including all the source codes, data, and data analyses, are available *(50).*

Selection and identification of food servings. This risk assessment included only food servings that contain meat and poultry and are RTE/PC. To select relevant foods, the Continuing Survey of Food Intake by Individuals, 1994–1996 and 1998 (CSFII) *(49)* was used to produce a list of 1,625 food codes describing all foods that contain meat or poultry. To determine the food codes that represented only RTE/PC products that could support growth of *C. perfringens,* the following exclusions were applied: (i) foods considered to be prepared from meat and poultry purchased raw; (ii) foods considered shelf stable (e.g., canned or dried); and (iii) foods with nitrite and ≥3% NaCl, based on lack of growth of vegetative cells under these conditions *(28).* The resulting 607 food codes represent 26,548 food servings. Using the fact that CSFII was a random survey with known population weights, the annual U.S. number of RTE/PC servings was estimated to be 69,600,000,000. Some fraction of the foods selected from the CSFII survey will not be RTE or PC. No survey information has been identified that could be used to estimate this fraction. It was assumed for this risk assessment that 80% of the servings selected, 55.7 billion servings, represent RTE/PC foods.

Level of type A CPE-positive *C. perfringens* vegetative cells in a serving. To estimate the level of type A *C. perfringens* enterotoxin (CPE)–positive *C. perfringens* vegetative cells in a serving before consumption, it is necessary to track both vegetative cells and spore populations, as spores may germinate into vegetative cells. Germination can result from the initial heat treatment or from cooking or reheating, or it may occur spontaneously. Model evaluation of the number N of *C. perfringens* type A CPE-positive vegetative cells present in the serving at the time of consumption is estimated as:

$$N = \left[\left\{\left[\left(\lfloor n_v G_c \rfloor + n_g\right) G_s\right] L_p + n_p\right] G_h\right] \quad (1)$$

where $\lfloor\ \rfloor$ indicates the floor function (next integer less than) and [ ] indicates the nearest integer function. The floor and nearest integer functions are used as a mathematical approximation to avoid using a stochastic growth model *(50).* The inputs to this equation are as follows: n_v is the number of type A CPE-positive vegetative cells present in the serving immediately after initial processing (before stabilization); n_g is the number of type A CPE-positive spores in the serving that germinate during storage; n_p is the number of type A CPE-positive spores in the serving that germinate during preparation, if the serving is hot-held; G_c is the growth factor for vegetative cell growth induced by the initial stabilization (cooling) regime (and by any other heating and cooling steps in initial processing); G_s is the growth or survival factor for vegetative cells occurring during storage and transport; L_p is the lethality factor for vegetative cells occurring during preparation; and G_h is the growth factor for vegetative cells during hot-holding (unity for non–hot-held servings).

Each of these inputs is obtained from analyses of available experimental data, using subsidiary models described below.

TABLE 1. *Prevalence and level of* C. perfringens *in meat products*

Reference	Season samples collected	Region	Lethality step	Presumptive colony confirmation	Products evaluated	Results
Kalinowski et al., 2003 *(28)*	Jan–Mar, May–June 2000	United States	Heated to 73.9°C	Yes[a]	Postlethality beef, pork, turkey	1% (2/197) samples with >0.5–2 log CP spores/g 0/197 samples with >2 log CFU/g
Taormina et al., 2003 *(46)*	Aug 2001–June 2002	Four midwestern facilities	75°C for 15 min	No	Postlethality beef, pork, chicken	2.5% (11/445) samples with 1.62 log spores/g
FSIS, 2003 *(52)*	27 Sept–17 Nov 2003	48 states and Puerto Rico	75°C for 20 min	Yes[b]	Ground beef samples from 546 processing plants	2/593 samples with 1 colony at the detection limit of 3 CFU/g.

[a] Presumptive *C. perfringens* colonies were confirmed via Gram stain, cell morphology, lactose fermentation, gelatin liquefaction, nitrate reduction, and motility reactions.

[b] Presumptive colonies were restreaked on tryptone-sulfite-cycloserine media and confirmed as *C. perfringens* by Gram stain followed by testing with API 20A kit (bioMerieux, Inc.) according to the manufacturer's instructions.

Number of *C. perfringens* vegetative cells and spores following heat treatment: n_v and n_s. *C. perfringens* spores and vegetative cells in RTE/PC products were assumed to be derived from the meat and spice components of the serving. Spices can contain substantial levels of *C. perfringens* spores *(10, 13, 34, 38)*. Addition of spices to raw commodities during the processing stage of RTE/PC foods could add to the *C. perfringens* spore load in the meat in such foods. The initial numbers of type A CPE-positive vegetative cells, n_v, and type A CPE-positive spores, n_s, present immediately after the initial heat treatment step (before stabilization) in a serving were estimated using the following:

$$n_v = P(wC_m f_m f_{vmA}) + \sum_j P(wC_{sj} f_{sj} f_{vsA})$$

$$n_s = P(wc_m f_m f_{smA}) + \sum_j P(wc_{sj} f_{sj} f_{ssA}) \quad (2)$$

where $P(z)$ denotes a Poisson sample with expected value z. The inputs here are as follows: w is the mass of serving; C is the concentration of vegetative cells immediately after initial processing (subscript m for meat, sj for spice of type j); c is the concentration of spores immediately after initial processing (subscript m for meat, sj for spice of type j); f_m, f_{sj} is the fraction of serving mass that is meat (f_m) or spice of type j (f_{sj}); f_{vmA}, f_{vsA} and f_{smA}, f_{ssA} are the fractions of vegetative cells present immediately after the initial lethality treatment, or spores germinating during storage and transport (first subscript v or s), and derived from meat or spice (second subscript m or s), that are type A, CPE positive.

Meat and poultry mass and spice mass: w, f_m, and f_{sj}. For each serving, CSFII provided the mass of the serving (w), the meat constituent mass fraction of the serving (f_m), and the mass fraction of the serving that is the spice indexed by j (f_{sj}).

Concentration of *C. perfringens* vegetative cells immediately after initial heat treatment: C_m. To estimate the vegetative cell concentrations (of all *C. perfringens* types) in RTE foods immediately after heat treatment, studies employing a heat treatment step and confirmation of presumptive *C. perfringens* colonies and performed in a meat and/or poultry matrix were identified (Table 1). Two studies, those of Kalinowski et al. *(28)* and FSIS *(52)*, satisfied all these criteria. Taormina et al. *(46)* did not confirm presumptive *C. perfringens* colonies, so the results of that study were used in the analysis as an upper bound on the cell concentration.

The few data in the above studies do not allow discrimination of the shape of the distribution of concentrations of vegetative cells to be expected in different meat samples. Therefore, the data of Greenberg et al. *(15)* were examined for qualitative evidence about the likely shape of the distribution. This study estimated the level of putrefactive anaerobic sporeformers (of which *C. perfringens* presumably made up some fraction) in ~2,400 heat-treated raw meat and poultry samples. The observed shape of the distribution at its upper end was consistent with that expected from a gamma distribution. Therefore, a gamma distribution shape was assumed for the concentration of *C. perfringens* spores in the meat and was used to analyze the selected studies.

PC foods are treated at temperatures that are lower than those used for RTE foods and not lethal for many *C. perfringens* vegetative cells. No measurements of *C. perfringens* vegetative cells in PC commodities are available, and any effect on vegetative cells or spores of sublethal temperatures in PC manufacture is not known. In the absence of any knowledge of modifying factors, it was assumed that the concentration of *C. perfringens* vegetative cells in PC servings is the same as that in raw meats. Three studies *(14, 45, 46)* were selected as representative and analyzed using the assumptions of a gamma distribution shape for the concentration distribution. The C_m notation is used for both RTE and PC foods.

Concentration of spores in the meat fraction: c_m. For RTE foods, standard industry heating of raw meat or poultry will activate a large fraction of the spores to germinate while killing vegetative cells that were present in the raw commodity. The concentration of remaining spores (that do not germinate) is represented by c_m. For PC foods, it was assumed that the initial processing step has no effect on spore concentrations in raw meats.

The value of c_m was obtained from studies that investigated the fraction of *C. perfringens* spores that remained inactivated following a heat treatment *(1, 7, 47, 56, 57)*. These studies showed a large variation in this fraction, depending on the heat treatment applied and on the strain of *C. perfringens*.

If the fraction of spores that germinate during heat treatment is η, then a fraction $(1 - \eta)$ remains as inactivated spores after the heat step applied to RTE foods, so the concentration, c_m, was estimated as:

$$c_m = \frac{1 - \eta}{\eta} C_m \quad (3)$$

for RTE foods (in which C_m corresponds to spores activated in

the heat step). For PC foods, the concentration c_m for a serving was estimated as:

$$c_m = C_{RTE}/\eta \quad (4)$$

where C_{RTE} corresponds to the distribution of C_m for RTE foods. As with C_m, the c_m notation is used for both RTE and PC foods, depending on the context.

To account for the large observed range of measurements for η, the varied heat treatments expected, and the variation in *C. perfringens* strains, η was modeled as varying from 5 to 75% (of the initial total number of spores) with a triangular distribution with a mode of 50% (and results were shown to be insensitive to these assumptions).

Fraction of *C. perfringens* spores and cells that are type A and CPE positive: f_{vmA}, f_{vsA}, f_{smA}, and f_{ssA}. To estimate the fraction of germinated spores and vegetative cells of *C. perfringens* that are type A and CPE positive, data from various studies *(9, 30, 40)* were analyzed with the assumption that these fractions were independent of the differing experimental conditions. It was not possible to distinguish the fractions between vegetative cells and germinating spores, but different estimates were obtained for meat and spices (the first three experiments listed used meat, and the last experiment used spices; the observations were consistent with the homogeneity assumption).

Concentration of germinated vegetative cells and spores in spices: C_{sj} and c_{sj}. All *C. perfringens* organisms found in spices are assumed to be spores. Four studies were selected as providing the most representative data on *C. perfringens* spore concentrations *(6, 35, 36, 38)*. Combining these with the spice data available from CSFII, it was possible to evaluate four "spices" with distinguishable concentration distributions: oregano; garlic; the combination of mustard, cumin, cinnamon, chili, cayenne pepper, and black pepper *(6, 36, 38)*; and all others combined *(35)*.

The experiments with spices were performed without a heat step, so presumably the total concentration of spores in the tested spices was underestimated. To account for this, an estimate, ϕ, of the fraction of spores that may germinate under favorable conditions without heat treatment was obtained. The ratio C_j/ϕ then estimates the initial concentration of spores in that spice, where C_j is the "as measured" concentration. The value of ϕ was estimated to be in the range of 1 to 10% *(1, 4, 7, 37, 47)* and was treated as a triangular distribution in this range with a mode of 5% (sensitivity analysis showed that the effect of this assumption is small).

For PC foods, the initial concentration of vegetative cells due to spores that germinate during initial processing, C_{sj}, is assumed to be equal to the "as measured" concentration (so $C_{sj} = C_j$). The remaining concentration of spores after initial processing is then given by $c_{sj} = (1/\phi - 1)C_j$. For RTE foods, the fraction η of spores that are activated by the initial processing is estimated and applied to the estimate for the initial concentration of spores, so that:

$$C_{sj} = \eta C_j/\phi \quad \text{and} \quad c_{sj} = (1 - \eta)C_j/\phi \quad (5)$$

Number of spores germinating during storage and consumer preparation: n_g and n_p. The number of type A CPE-positive spores, n_g, in a serving that spontaneously germinate during storage (and, if this serving is hot-held, the number of spores, n_p, that subsequently germinate during preparation) was estimated as:

$$n_g = B(n_s, g_s)$$
$$n_p = B\{[(n_s - n_g)l_s], g_p\} \quad (6)$$

where $B(m, z)$ represents a binomial sample with probability z from a sample of size m, and the [ ] symbol indicates the nearest integer function. The nearest integer function implements an approximation that avoids using a stochastic death model for cells *(50)*. The inputs are as follows: n_s is the number of type A CPE-positive spores in the serving immediately after initial processing (see above); g_s is the fraction of spores that germinate during storage and transport; l_s is the lethality factor for spores during storage and transport; and g_p is the fraction of spores that germinate during preparation.

Fraction of spores that germinate during storage and transport: g_s. Following a heat treatment, a large fraction of *C. perfringens* spores can germinate; however, those spores that did not germinate could germinate spontaneously at a later point, a phenomenon that has been observed in multiple studies under many conditions *(1, 4, 7, 37, 47)*. To encompass the range of observations, the fraction g_s of type A CPE-positive strains germinating in storage was modeled using a triangular distribution ranging from 0 to 5%, with a mode of 2.5% (results were shown to be insensitive to these estimates using sensitivity analysis).

Lethality factor for spores during storage and transport: l_s. Spores are not greatly affected by refrigeration and freezing *(4, 5, 42)*, although vegetative cells are sensitive to freezing. In this risk assessment, spores are assumed to be unaffected by any temperature encountered during storage, so that the lethality factor l_s is assumed to be unity.

Fraction of spores that germinate during consumer preparation (reheating): g_p. Spores in RTE products may germinate during consumer cooking (reheating) and become vegetative cells that can grow during a hot-holding period unless they are held at a sufficiently high temperature. One study *(56)* that effectively measured g_p relative to η (the fraction of spores that germinate on first heat treatment) was identified. *C. perfringens* spores were heat treated using a regime that was near-optimal for stimulating germination for the strain tested *(12)*, followed by a second heat treatment to estimate the fraction of spores remaining from the first heat treatment. Since actual heat treatments are unlikely to be optimal for all strains simultaneously, g_p was conditioned on η, i.e., it is treated as variable from 0 to $(0.75 - \eta)/(1 - \eta)$. This is the upper limit corresponding to the assumption that there is an upper bound of 75% on the total fraction of spores that might be activated by up to two heat treatments, based on experimental studies measuring maximum absolute germination fractions in single heat treatments, with a triangular distribution with a mode halfway between zero and the upper limit. Sensitivity analysis showed only a small effect from varying these assumptions (this primarily affects the estimate of illnesses due to hot-holding).

Growth factor for vegetative cells induced by the initial stabilization (cooling): G_c. The amount of *C. perfringens* growth allowed during stabilization is modeled as an input to the risk assessment to estimate the effect of different stabilization performance standards. Ideally, to model this effect would require knowledge of a mapping between the regulatory level of growth allowed and the distribution of the amount of growth achieved in practice in all RTE/PC. This information is not currently available, so the following approach was taken. In the implementation of the model, the option of specifying any variability distribution for growth is provided. Thus, it is possible to specify a single value for the growth of *C. perfringens* in all RTE/PC or a distribution of values corresponding to the possible range of values that would be achieved in practice for a given regulation. In the current application, fixed values of growth, G_c, were chosen (log G_c equal

to 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, or 3.5) to estimate the human health impact of altering the current stabilization performance standard.

Growth or survival of vegetative cells during storage: G_s. The factor G_s is obtained as the product of two factors, one for each period of storage (manufacturer to retail and retail to consumer storage). The factor for each period is obtained by applying the respective growth or death rate for the corresponding temperature and time scenarios as discussed below.

Growth rates are estimated using primary and secondary growth models. A primary growth model (with growth rate as one of its parameters) was used to fit measured growth of *C. perfringens* as a function of time at fixed temperatures. Data on measured cell densities as a function of time at fixed temperatures were obtained *(18, 20, 31)* for the experiments described by Juneja et al. *(27)* involving broth, for those described by Juneja et al. *(26)* involving cooked cured beef, for those described by Juneja and Marks *(22)* involving cooked cured chicken, and for those described by Huang *(17)* involving cooked ground beef. These were simultaneously fitted to the growth model to obtain best estimates of the parameters, including a between-experiment variability, together with uncertainty estimates.

The secondary models describe how the growth rate and delay time of the primary model vary with temperature, a variation typically being fitted by a model of Ratkowsky form. The secondary model for growth rates obtained for cooked cured beef and cooked cured chicken (which gave growth rate estimates that were statistically indistinguishable) was judged most representative for application to RTE/PC foods in this risk assessment. However, this model was then modified by an additional factor (with attendant uncertainty distribution) to obtain agreement with a compilation of 174 further values for growth rate (for temperatures varying from 12 to 51°C) reported in published literature *(50)*.

The growth rate of *C. perfringens* can be influenced by factors other than temperature (e.g., NaCl, nitrite, pH, water activity, and oxygen); however, only the effect of salt, nitrite with salt, pH, and water activity on growth could be quantified from the available data. To estimate the effect of low salt concentrations in food on the growth of *C. perfringens* in RTE/PC, the data of Kalinowski et al. *(28)* and Juneja et al. *(25)* were used. For foods containing nitrite with salt concentrations of ≥3%, no *C. perfringens* growth is assumed *(28)*. For foods containing low salt and nitrite, a slower *C. perfringens* growth rate was calculated. Extensive experimental evidence for growth of *C. perfringens* in broth *(25)* showed that the growth rate decreased quadratically with salt concentration; and the variation obtained was consistent with the variation in growth rate with salt content observed by Kalinowski et al. *(28)* in a few experiments in cured and uncured turkey. The effect of salt (the concentration of which was available for the CSFII servings used) was incorporated using the quadratic variation observed by Juneja et al. *(25)*. For nitrite, for which only presence or absence could be inferred for the CSFII servings, the data of Kalinowski et al. *(28)* were used to estimate a single reduction factor to be used for growth rate when nitrite was present. Juneja et al. *(25)* showed a significant effect of initial pH on lag phase duration and generation time for *C. perfringens* in a laboratory broth medium. However, analysis of the published exponential growth rates, corresponding to the growth rate used in the risk assessment, showed no effect of pH over the range of values measured. No variation of growth rate with pH was incorporated. Experiments by Kang et al. *(29)* indicated little variation of *C. perfringens* growth rate at a water activity of >0.95, the range expected for RTE/PC foods included in the risk assessment, so no effect of water activity was included.

Maximum cell densities observed in various food matrices were 7.6 log in cooked cured beef *(26)*, 8.07 log in cooked cured chicken *(22)*, and 8.03 log in cooked ground beef *(17)*. To encompass the differences observed in the studies, the maximum cell density for *C. perfringens* in RTE/PC is assumed to be 8 log, with a variability of 0.5 log. The effect of this assumption was addressed using sensitivity analysis.

To estimate a death rate for vegetative *C. perfringens* at refrigeration and freezing temperature, data from several studies using food as a matrix were identified *(4, 21, 24, 28, 44, 46)*. In all the studies examined, concentrations of *C. perfringens* decreased during storage in a way that was consistent with a regular exponential decrease with time. Death rates were estimated for different temperature regimes from these experiments, although the effects of "cold shock" were omitted. In this risk assessment, it is assumed that the cutoff temperature point for growth is the lowest temperature estimated for growth from the secondary growth model (~12.5C). Below that temperature, *C. perfringens* vegetative cells are assumed to die, and above that temperature they are assumed to grow.

Lethality factor for vegetative cells occurring during preparation: L_p. During preparation of RTE/PC, some products may be consumed cold, while others may be reheated for immediate consumption. Alternatively, some reheated food items may be hot-held for consumption at a later time. During reheating, the model predicts that as the temperature rises above about 53.5°C, destruction of vegetative cells will occur.

The destruction of vegetative cells at high temperatures was characterized by the length of time taken for the concentration of vegetative cells to decrease by a factor of 10 (*D*-value). Experimental evidence on *D*-values for *C. perfringens* vegetative cells was collected and analyzed *(23, 39, 41)*. Examination of the *D*-values indicated that they could be classified into two classes. The first class comprises those obtained from experiments in which there was a substantial heat shock (*C. perfringens* was cultivated at constant temperatures of 37 to 45°C, followed by determination of *D*-value at a temperature higher by 15°C or more than the cultivation temperature). The second class comprises those obtained from experiments in which there was a minimal heat shock (*C. perfringens* was cultivated at temperatures higher than 45°C or with the temperature increasing at a constant rate before determination of the *D*-value). The variation of *D*-values with temperature for these two classes was modeled, and these models were fitted to available data. In both cases the *D*-value decreased exponentially with temperature, but with different coefficients for the two classes.

For this risk assessment, these two classifications of reheating were applied to microwave cooking (large heat shock) or oven cooking (lesser heat shock). No consumer preparation survey data were identified that provided information on the times for which foods are heated or the time elapsed before consumption. It is assumed that 50% of RTE/PC products are heated rapidly, as in a microwave oven, reaching the final temperature in a time that is uniformly distributed from 1 to 10 min. The other 50% of RTE/PC products are assumed to be cooked as in an oven, with cooking times varying uniformly for 10 to 30 min. To estimate the distribution of temperatures of reheating, the risk assessment used a nationwide survey conducted by Audits International/FDA *(3)*.

Growth factor for vegetative cells during hot-holding: G_h. Vegetative cells already present in the food or spores newly germinating during reheating may proliferate in hot-held food at or below the maximum growth temperature, estimated to be 53.5°C. For this risk assessment, it is assumed that hot-held food is ini-

tially heated sufficiently to activate spores and kill all vegetative cells present. Subsequently growth is assumed to proceed according to the primary growth model for the temperature of hot-holding.

Duration and temperature. Growth and mortality of *C. perfringens* are dependent on time and temperature. At the retailer, the storage time for nonfrozen and frozen products is assumed to be uniformly distributed between 10 and 30 days *(53)*. The storage temperature distribution reached at retail for nonfrozen and frozen foods is assumed to be represented by Audits International/FDA survey data.

Data from the same survey were used to estimate a distribution of product temperatures during consumer storage, and survey data collected by the American Meat Institute *(2)* were used to estimate a distribution for storage times.

To estimate the distribution of hot-holding temperature, food holding temperature data were obtained during a survey of risk factors that contribute to foodborne illness *(55)* including whether hot-holding temperatures were in or out of compliance with 1997 FDA Food Code requirements *(54)*.

No data on the duration of hot-holding were located. The 1997 FDA Food Code *(54)* sets no time limit on hot-holding, although a minimum temperature of 60°C is required in food establishments. It was assumed that the period varies from 0.5 to 5 h, with a probability density that decreases linearly to zero at 5 h.

Dose-response relationship. The purpose of the dose-response in this risk assessment was to provide an estimate of the probability of human diarrheal illness following ingestion of a specified number of *C. perfringens* vegetative cells. To develop such a relationship, information from four *C. perfringens* human feeding studies were used *(8, 11, 16, 43)*. The data from these studies that were included involved the administration of type A and CPE-positive *C. perfringens*.

None of the available data are adequate to define a shape (functional form) for the dose-response relationship for *C. perfringens*, so the simplest biologically plausible dose-response function, the linear-exponential, was chosen. For this functional form the probability of illness (diarrhea) given a dose of d, $P(d; k)$, is

$$P(d; k) = 1 - \exp(-kd) \quad (7)$$

where k may be interpreted as the virulence of the particular *C. perfringens* isolate administered. However, there is no evidence that the virulence is the same for different isolates of even the same strain of *C. perfringens*, so this dose-response shape refers to isolate-specific dose-response. The available data indicate that the variation in virulence between isolates encompasses orders of magnitude, as indicated by the raw data in Figure 2. The distribution of maximum likelihood estimates for k obtained for the individual isolates in different feeding experiments was examined and found to be indistinguishable from lognormal ($P = 0.79$, Shapiro-Wilk test—this is a heuristic use of the test; the P value obtained is not accurate, but large values indicate nonrejection). The variation of virulence for causing human diarrhea between isolates of *C. perfringens* was therefore modeled as a lognormal distribution. Figure 2 illustrates the effective isolate-averaged dose-response curve (solid line; this is the probability for a response to a random isolate) together with individual isolate dose-response curves at the median and 95% confidence limits for individual-isolate virulence (dotted lines). The variation between isolates is sufficiently large that identification of the exact shape of the individual-isolate dose-response curve is much less important than accounting for the variability in virulence between different isolates of *C. perfringens*. The effective dose-response curve (probability for diarrhea versus number of ingested cells) for fixed numbers of cells of an arbitrary isolate of *C. perfringens* corresponds to the convolution of the within-isolate (linear-exponential) dose-response and the between-isolate (lognormal) variation. The latter variation is so large that the assumed shape for the individual-isolate dose-response curve has little impact. This was confirmed by repeating the entire analysis using a probit-log-dose shape for the individual isolates and finding practically indistinguishable results.

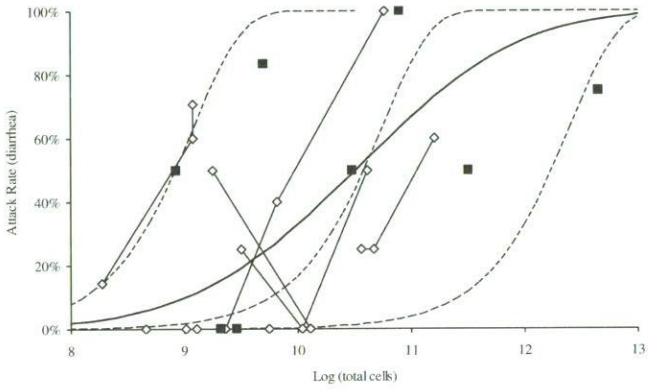

FIGURE 2. *Individual isolate dose-response curves (dotted) at the median and 95% confidence limits on the distribution for isolates and the isolate-averaged dose-response curve (solid), superposed on experimental data. Points joined by lines indicate multiple-dose experiments for a single* C. perfringens *isolate, while single points are for single-dose experiments with multiple* C. perfringens *isolates.*

RESULTS AND DISCUSSION

The risk assessment model was run with multiple fixed values of *C. perfringens* growth during stabilization, in order to evaluate the effect of variation in growth during stabilization on the estimates of annual *C. perfringens* illnesses. Table 2 shows how two estimators of risk per serving vary as the growth during stabilization increases from 0.5 to 3.5 log. The range in the median estimate for rate of illness is from approximately 1.3 illnesses per million servings to 2.7 illnesses per million servings. The total number of servings of RTE/PC foods in the United States per year was estimated to be approximately 55.7 billion, so these estimates correspond to a range from 74,000 to 149,000 cases per year for 0.5- to 3.5-log growth, respectively (using a curve fit to the median estimates).

Currently, there is no active or passive surveillance reporting system for *C. perfringens* illnesses. Most detected illnesses are due to outbreaks. Mead et al. *(33)* estimated approximately 250,000 cases of *C. perfringens* food poisoning annually from outbreaks caused by all food sources, so illness attributable to RTE/PC should be a fraction of this total. The Mead et al. *(33)* methodology, however, required considerable extrapolation (a factor of 380) from the number of reported outbreak illnesses to the total number of illnesses. In addition, based on case investigations of a small fraction of the illnesses reported, most such illnesses are attributed to consumption of meat and poultry purchased as raw products and not as RTE/PC. Assuming that

TABLE 2. *Estimates for annual numbers and rate of illnesses from* C. perfringens *in RTE/PC meat and poultry products*

Growth (log)	Annual no. of illnesses (55.7 billion servings)			Rate per million servings		
	MLE estimate[a]	Median estimate[b]	Curve fit[c]	MLE estimate	Median estimate	Curve fit
0.5	74,000	75,000	74,000	1.33	1.34	1.34
1	82,000	78,000	79,000	1.47	1.40	1.42
1.5	89,000	89,000	86,000	1.59	1.59	1.54
2	97,000	93,000	96,000	1.74	1.67	1.72
2.5	101,000	108,000	108,000	1.82	1.95	1.95
3	117,000	128,000	126,000	2.10	2.29	2.26
3.5	137,000	148,000	149,000	2.46	2.66	2.68

[a] One billion servings simulated at each growth, with all parameters set at the maximum likelihood for uncertainty.
[b] Geometric mean of 600 values for each growth, with each value corresponding to an uncertainty simulation of 30 million servings.
[c] The best-fit curve to the median estimate, taking account of uncertainties.

federally inspected plants are meeting the current 1-log stabilization performance standard, the 79,000 illnesses at 1-log growth obtained here by modeling falls within the Mead et al. estimate. This suggests that the model may reasonably predict the annual number of *C. perfringens* illnesses due to consumption of RTE/PC foods; however, there are no available epidemiologic data to validate the model estimates. In addition, the model was developed more to evaluate the relative effect of changes of the stabilization performance standards than to predict the absolute number of illnesses.

The major fraction of illnesses (91%) predicted by the model is due to growth during retail and consumer storage. If the storage temperature is below the minimum temperature for growth (~12.5°C), illness is unlikely. If it is above this temperature, the length of storage is usually sufficiently long that any initial numbers of *C. perfringens* vegetative cells are predicted to grow to stationary phase. As a result, illness becomes more likely. The available data indicate that a small fraction of refrigerators have such high temperatures *(3)*. This was confirmed by a more recent consumer storage survey *(19)*. This risk assessment did not evaluate the possibility that psychrotrophic bacteria would likely be present in RTE/PC products and could competitively outgrow *C. perfringens* at low temperatures. Such a phenomenon would have the effect of reducing the total number of predicted *C. perfringens* foodborne illnesses.

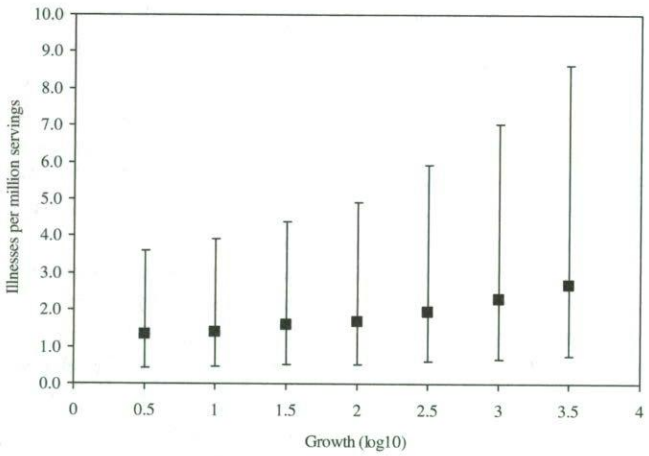

FIGURE 3. *Uncertainty estimates for rate of diarrhea for fixed growth during stabilization (90% confidence intervals for modeled uncertainties). The uncertainty distributions are correlated between allowed growths and should be interpreted as showing the range of uncertainty for the complete curve of illness rate versus allowed growth.*

It follows that *C. perfringens* growth during stabilization of RTE/PC meat and poultry products has a small overall effect on the likelihood of illness. At 1-log allowable growth during stabilization, the model estimates that approximately 0.05% of *C. perfringens* illnesses are due to growth during stabilization. However, as more growth is allowed during stabilization, a larger fraction of illnesses can be caused by concentrations of cells that arise entirely due to that growth (with no further growth during retail and consumer storage).

Growth during retail and consumer storage is the major predicted cause of illnesses from *C. perfringens* in RTE/PC meat and poultry products. This indicates that the principal determinants of illness are the initial concentrations (prevalence and count) of *C. perfringens* in servings, the distribution of storage temperatures, the distribution of times during storage, and the maximum concentration of *C. perfringens* in the serving. Other factors, such as *C. perfringens* vegetative cell death rates during cold storage, have very little effect on the likelihood of illness. Even the *C. perfringens* growth rate achieved at temperatures close to its lowest growth temperature (about 12.5°C) is unimportant so long as the temperature is sufficiently high (above 12.5°C) that a large amount of growth can occur during typical storage times.

The uncertainty of the results is illustrated by Figure 3, which shows the median estimate and the modeled 90% confidence interval for the rates of illness for fixed *C. perfringens* growth during stabilization for seven such growths between 0.5 and 3.5 log. These uncertainty estimates represent uncertainties that could be determined empirically from available data; they do not include systematic or model error estimates. They correspond to a geometric standard deviation of approximately 2.

This risk assessment also estimated the fraction of *C.*

perfringens illnesses due to improper hot-holding. The risk assessment model estimates that about 6,000, or 7.6%, of the total 79,000 illnesses are due to improper hot-holding. It is likely that this is an underestimate because the model treats each serving as independent. Effectively, each illness attributed by the model to improper hot-holding may represent multiple illnesses from one hot-holding event, since hot-held food servings can be heated together and cross-contaminate other servings. There is no independent estimate of the fraction of *C. perfringens* illnesses contributed by improper hot-holding from RTE/PC foods; nonetheless, the result suggests that improper hot-holding does contribute to the annual burden of *C. perfringens* illnesses and is likely a risk factor of consumption of RTE/PC products.

This risk assessment was developed to estimate the impact to public health from *C. perfringens* given various policy options for cooling (stabilization) standards for RTE/PC products. As such, the model is a tool to evaluate the effect of interventions, rather than one to predict the absolute number of illnesses from RTE/PC foods contaminated with *C. perfringens*. The risk assessment model predicted that allowing a 10- or 100-fold increase in the 1-log maximal allowable growth of *C. perfringens* results in a 1.2- or 1.6-fold increase, respectively, in illnesses caused by *C. perfringens*. Improper retail and consumer refrigeration accounted for the majority of the predicted *C. perfringens* illnesses, while stabilization accounted for less than 1% of illnesses. Therefore, efforts to reduce illnesses from *C. perfringens* in RTE/PC meat and poultry products should focus on retail and consumer storage and preparation methods. Complete details of this risk assessment, including the full-length report, the risk assessment model, and itemized responses to external peer reviewer and stakeholder comments are available through the FSIS Web site at http://www.fsis.usda.gov/Science/Risk_Assessments/index.asp.

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